Multiple Endocrine Neoplasia 1 Gene Alterations in MEN1-Associated and Sporadic Lipomas

Vortmeyer, Alexander O ; Pak, Evgenia ; Pack, Svetlana ; Böni, Roland ; Zhuang, Zhengping

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CORRESPONDENCE

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Multiple endocrine neoplasia 1 (MEN1) syndrome is characterized by the development of parathyroid adenomas, pituitary adenomas, and duodenal and/or pancreatic neuroendocrine tumors. The vast majority, and possibly all, of these tumors are characterized by two genetic hits—germline mutation of the MEN1 tumor suppressor gene combined with allelic deletion of the corresponding wild-type allele *(1).* Alterations of the MEN1 gene are not only found in MEN1-associated tumors but also, with decreasing frequency, in sporadic neuroendocrine tumors of the foregut *(2,3),* parathyroid tumors *(4),* and pituitary adenomas *(5).*

Lipomatous tumors are known to occur in a relatively high proportion of patients with MEN1 disease. In this study, we attempted to determine the role of the MEN1 gene in the development of MEN1-associated and sporadic lipomas. For genetic tissue analysis of MEN1-associated lipomas, we obtained preparations by touching tumor tissue to a glass slide to transfer a limited number of cells; we then performed fluorescence *in situ* hybridization (FISH) on such touch preparations to detect deletions of the MEN1 wild-type allele in two tumors that were excised from two patients with known MEN1 gene germline mutations (case 1—a 61-year-old male with an abdominal wall lipoma, mutation status L22R; case 2—a 69-year-old male with a lipoma of the right thigh, mutation status W436R). FISH was performed using as a probe cosmid clone c10B11 (size, 40 kilobases), which contains the MEN1 gene *(6).* For analysis of sporadic lipomas, we performed polymerase chain reaction (PCR)-based single-strand conformation polymorphism and sequence analysis using formalin-fixed, paraffin-embedded tissue.

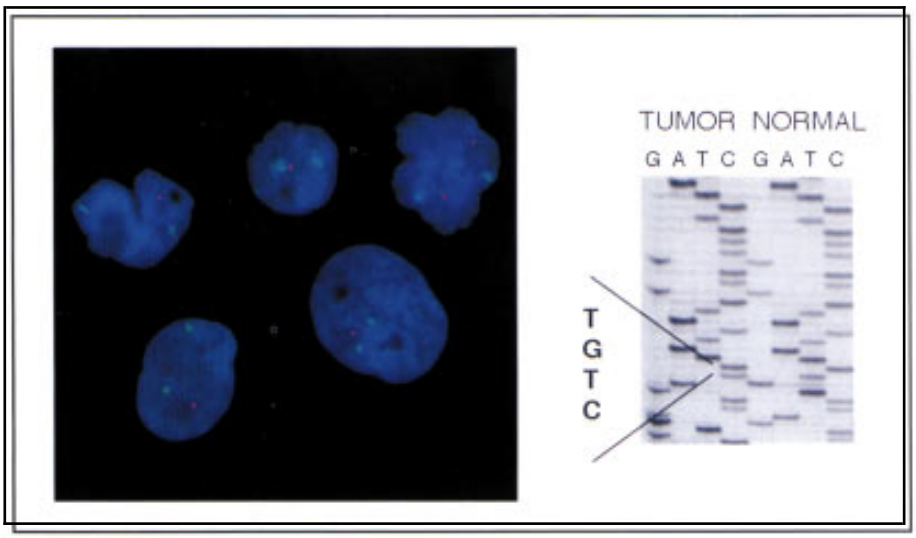

Fig. 1. Detection of genetic alterations in multiple endocrine neoplasia 1 (MEN1)-associated and sporadic lipomas. **Left panel**) fluorescence *in situ* hybridization analysis of a preparation, obtained by touching tumor tissue to a glass slide to transfer a limited number of cells, from lipomatous tissue (case 1) using cosmid clone c10B11, which contains the MEN1 gene as a probe; red signals = the presence of the MEN1 gene on chromosome 11q13, green signals = the centromeric region of chromosome 11; four cells exhibit loss of one MEN1 allele; one cell in the right upper corner shows the presence of both MEN1 gene copies, and therefore is interpreted as a non-neoplastic stromal cell. **Right panel**) Sequence analysis of a sporadic lipoma (case 1) reveals a TGTC deletion in the tumor cells when compared with normal control tissue from the same patient.

FISH analysis of the two lipomas, which were excised from patients with known MEN1 disease, revealed loss of one MEN1 allele in 53% of the cells examined from case 1 (Fig. 1, left panel) and in 63% of the cells examined from case 2. It appears from this finding that the lipoma cells are affected by genetic deletion, whereas both MEN1 gene copies were visualized in normal cellular constituents. Furthermore, in both cases, two copies of the chromosome 11 alpha satellite, located in the centromere, were present in all of the cells, as shown by a control probe. In conjunction with a recent PCR-based deletion analysis of three lipomas *(7),* our results confirm the hypothesis that mutation of the MEN1 gene and subsequent loss of the wild-type allele are associated with or causative for the development of lipomatous tumors in patients with MEN1 disease.

To investigate the role of the MEN1 gene in sporadic lipomas, we analyzed six sporadic tumors (female : male = 2:4; mean age = 33 ± 16 years). Lipomatous and normal control tissue was scraped from sections of paraffin-embedded tissue after deparaffinization in xylene and alcohol. The removed tissue was placed in proteinase K buffer for DNA extraction. The DNA samples were PCR amplified with 13 pairs of markers amplifying the coding regions of the MEN1 gene, including exons 2–10, as previously described *(2).* The amplification products were visualized after polyacrylamide gel electrophoresis. Due to poor tissue quality, amplification products were only obtained for exons 2, 3, 8, and 9. In one case, single-strand conformation polymorphism analysis and subsequent sequence analysis revealed a four-nucleotide deletion in exon 2 (Fig. 1, right panel). This deletion was present only in the tumor tissue but not in the normal tissue control from the same patient. From this finding, we conclude that MEN1 gene mutation may play a role not only in the development of MEN1-associated lipomas but also in sporadic lipomas.

ALEXANDER O. VORTMEYER
ROLAND BÖNI
EVGENIA PAK
SVETLANA PACK
ZHENGPING ZHUANG

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Notes

Affiliations of authors: A. Vortmeyer, E. Pak, S. Pack, Laboratory of Pathology, Division of Clinical Sciences, National Cancer Institute, Bethesda, MD; R. Böni, Department of Dermatology, University Hospital, Zurich, Switzerland.

Correspondence to: Zhengping Zhuang, M.D., Ph.D., National Institutes of Health, Bldg. 10, Rm. 2N212, Bethesda, MD 20892.

Re: Prevalence of Cancer

A recent Stat Bite *(1)* showed the prevalent cases of eight cancer types in the U.S. population. Practitioners of public health often ignore prevalence as a measure of disease frequency, probably because differences in prevalence between groups can arise from differences in incidence or from differences in the average survival *(2).* While this ambiguity may cloud etiologic interpretation of differences in prevalence, the mixture of occurrence and survival makes prevalence an important measure of the distribution of disease.

Fig. 1 shows our estimates of the prevalence, prevalent cases, and annual incident cases of breast cancer by age among U.S. women in 1997. We estimated the prevalence by applying the method of Alho *(3)* to data reported by the National Cancer Institute (NCI) *(4),* the National Center for Health Statistics, and the Census Bureau. A further description is available from the authors.

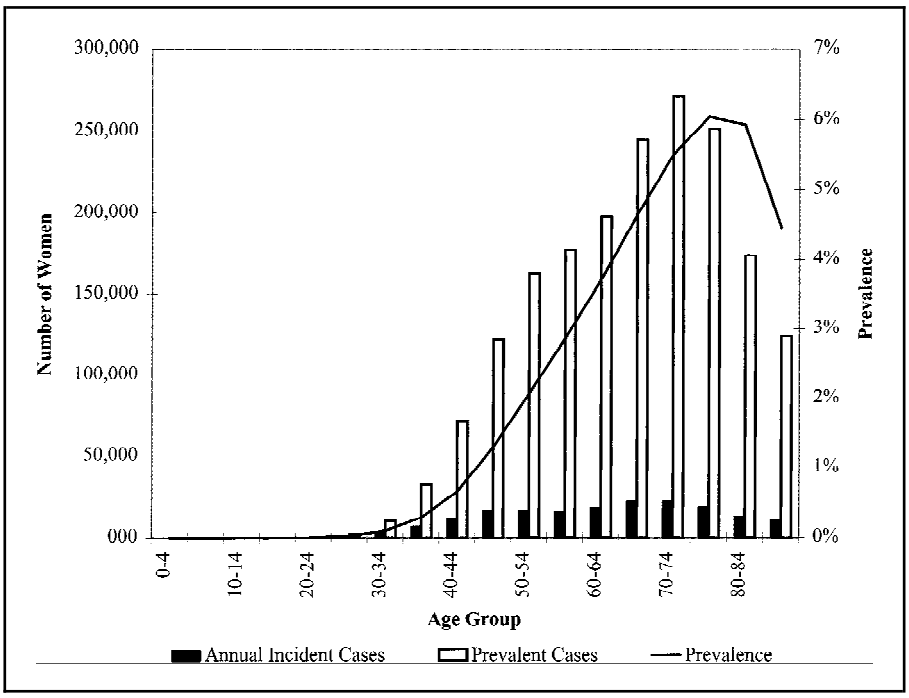

Fig. 1. Estimated prevalence, number of prevalent cases, and annual number of incident cases of breast cancer among U.S. women in 1997.

The age-specific prevalence provides insight into the distribution of breast cancer among U.S. women. This insight differs from what one would learn by examining incidence rates alone. For example, while about 22% of incident cases of breast cancer occur in women younger than age 50 years, only about 12% of the prevalent cases exist in that group. Similarly, 51% of new cases occur in women younger than age 65 years, but only about 42% of the prevalent cases exist in that group. The ratio of the number of prevalent cases to annual incident cases increases in older age groups. Among women 40–44 years old, the ratio of prevalent cases to annual incident cases is about 6 to 1. Among women 70–74 years old, the ratio is about 12 to 1.

This description of prevalent breast cancer emphasizes the importance of including the elderly population in studies of cancer survivors. There are about as many prevalent cases of breast cancer among women ages 80–84 years as there are among women ages 55–59 years. Women may weigh aspects of cancer treatment and survival differently depending on their age. For example, younger women are often concerned about the effect of treatment on their ability to meet their obligations, such as caring for family members *(5).* About 20% of the women less than 75 years old at diagnosis said this was a very important consideration in making decisions about treatment for breast cancer. Among women age 75 years and older, 7% said this was a very important consideration and 83% said it was not important at all. Younger women may weight most heavily the expected duration of survival, whereas older women may weight most heavily the quality of their expected survival, particularly their ability to live independently. The elderly have often been excluded from studies of treatment efficacy, and the frequency of age-related exclusion has increased in recent years *(6),* at least in the case of acute myocardial infarction. As the NCI embarks on a formal program to study cancer survivors *(7),* the program must take care to avoid a reprise of this ageist history.

TIMOTHY L. LASH
REBECCA A. SILLIMAN

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